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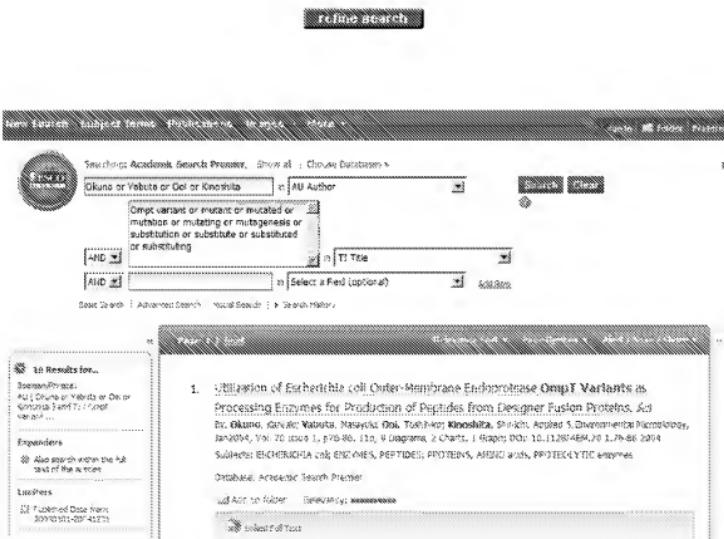


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Adv Enzymol. 2004 Jan;70:76-86

## Utilization of *Escherichia coli* outer-membrane endoprotease OmpT variants as processing enzymes for production of peptides from designer fusion proteins.

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### Abstract

*Escherichia coli* outer-membrane endoprotease OmpT has suitable properties for processing fusion proteins to produce peptides and proteins. However, utilization of this protease for such production has been restricted due to its generally low cleavage efficiency at Arg (or Lys)-Xaa, where Xaa is a nonbasic N-terminal amino acid of a target polypeptide. The objective of this study was to generate a specific and efficient OmpT protease and to utilize it as a processing enzyme for producing various peptides and proteins by converting its substrate specificity. Since OmpT Arg-97 is proposed to interact with the P1' amino acid of its substrates, OmpT variants with variations at Asp(97) were constructed by replacing this amino acid with 19 natural amino acids to alter the cleavage specificity at Arg (P1)-Xaa (P1'). The variant OmpT that had a methionine at this position, but not the wild-type OmpT, efficiently cleaved a fusion protein containing the amino acid sequence -Arg-Arg-Arg-Ala-Arg downward arrow motif, in which motilin is a model peptide with a phenylalanine at the N terminus. The OmpT variants with leucine and histidine at position 97 were useful in releasing human adrenocorticotrophic hormone (1-24) (serine at the N terminus) and human calcitonin precursor (cysteine at the N terminus), respectively, from fusion protein. Motilin was produced by this method and was purified up to 99.0% by two chromatographic steps, the yield was 150 mg/liter of culture. Our novel method in which the OmpT variants are used could be employed for production of various peptides and proteins.

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However, utilization

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acid of a target polypeptide. The objective of this study was to generate a

specific and efficient OmpT protease and to utilize it as a processing enzyme for

producing various peptides and proteins by converting its substrate specificity.

Since OmpT Asp(97) is proposed to interact with the P1' amino acid of its substrates, OmpT variants with variations at Asp(97) were constructed by replacing this amino acid with 19 natural amino acids to alter the cleavage specificity at Arg (P1)-Xaa (P1'). The variant OmpT that had a methionine at this position, but not the wild-type OmpT, efficiently cleaved a fusion protein containing the amino acid sequence -Arg-Arg-Arg-Ala-Arg downward arrow motilin, in which motilin is a model peptide with a phenylalanine at the N terminus. The OmpT variants with leucine and histidine at position 97 were useful in releasing human adrenocorticotrophic hormone (1-24) (serine at the N terminus) and human calcitonin precursor (cysteine at the N terminus), respectively, from fusion proteins. Motilin was produced by this method and was purified up to 99.0% by two chromatographic steps; the yield was 160 mg/liter of culture. Our novel method in which the OmpT variants are used could be employed for production of various peptides and proteins.

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## Utilization of *Escherichia coli* Outer-Membrane Endoprotease OmpT Variants as Processing Enzymes for Production of Peptides from Designer Fusion Proteins

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